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Award Number: W81XWH-08-2-0132

TITLE: MISSION CONNECT MILD TBI TRANSLATIONAL RESEARCH CONSORTIUM

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REPORT DATE: August 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

■ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-08-2010	Annual	1 AUG 2009-31 JUL 2010

4. TITLE AND SUBTITLE
MISSION CONNECT MILD TBI TRANSLATIONAL RESEARCH CONSORTIUM

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-2-0132

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES):
Baylor College of Medicine

Houston, TX 77030

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During 02 year, the Model or Neuroprotection Working Groups met on 18 November 2009, 11 December 2009, 17 December 2009, 20 January 2010, 23 March 2010 and 22 April 2010. At the 20 January, 2010 meeting, it was decided that the Model and Neuroprotection Working Groups would merge since many of the members of the MWG also were members of the Neuroprotection Working Group. Prior to each meeting after the groups merged, Drs. DeWitt and Perez-Polo, the Chairs of the Model Working Group and Neuroprotection Working Group, respectively, will work together to compile an agenda that meets the needs of both groups. This report is a compilation of the results of the m eetings of the combined committees.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER	USAMRMC
U	U	U	UU	88	

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INTRODUCTION

During 02 year, the Model or Neuroprotection Working Groups met on 18 November 2009, 11 December 2009, 17 December 2009, 20 January 2010, 23 March 2010 and 22 April 2010.

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BODY OF REPORT

Model/Neuroprotection Working Group Members:

Pramod Dash, Ph.D.
Douglas DeWitt, Ph.D., Co-Chair
Raymond Grill, Ph.D.
Claire Hulsebosch, Ph.D.
John Holcomb, M.D.
Thomas Kent, M.D.
Harvey Levin, Ph.D.
Pon Narayana, Ph.D.
Regino Perez-Polo, Ph.D., Co-Chair
Matthew Rasband, Ph.D.
Claudia Robertson, M.D.

M/NWG Goals:

1. To ensure that a standard protocol is developed and used consistently for each of the experimental models used by all Mission Connect Translational MTBI Translational Research Consortium investigators.

2. To thoroughly characterize the models by establishing and performing a standard group of physiological, histopathological and behavioral outcome measures on all four models.

3. To evaluate the efficacy of the neuroprotective agents that are being tested during the current period of support of the Mission Connect Translational MTBI Translational Research Consortium and to identify potentially neuroprotective agents or strategies for future testing.

The table below is a summary of the experimental traumatic brain injury (TBI) models that are used by the investigators of the Mission Connect Translational MTBI Translational Research Consortium. The 1^{o} Investigator is investigator who developed the experimental model and/or is providing injured animals for the Investigators who are studying the effects of that type of TBI.

Mission Connect mTBI Consortium experimental TBI models		
Model	**1^{o} Investigator**	**Investigators**
Fluid percussion injury	DeWitt	Dash, DeWitt, Perez-Polo, Grill

Controlled cortical impact	Robertson	Robertson
Rasband blast injury	Rasband	Rasband, Grill, Dash
Vandenberg blast injury	DeWitt	DeWitt, Grill, Dash, Perez-Polo

mTBI Model Standardization

Based on discussions during the meetings, the MWG recommended that the following data will be acquired for all of the experimental TBI models:

1. Mean arterial blood pressure & heart rate
2. Cerebral perfusion (e.g. laser Doppler flowmetry, ^{14}C-iodoamphetamine autoradiography
3. Vestibulomotor outcome (e.g. beam balance/beam walking, RotaRod performance)
4. Latency to return of the righting reflex (a measure of duration of unconsciousness after injury)
5. Spatial or working memory function (e.g Morris water maze or Barnes maze performance)
6. Anxiety (e.g. startle reflex, open-field behavior)
7. Histopathological outcome (e.g. counts of surviving neurons in cortex and hippocampus)
8. Blood brain barrier permeability (albumin and/or dextrans of different sizes)
9. APP, BDNF, GFAP immunohistochemistry.

These data will be used to characterize, compare and contrast the pathophysiological effects of the experimental TBI models. In addition, each investigator may make additional measurements relevant to his/her specific project.

The table of assignments for the model characterization studies was completed (below).

Measurement	Model	Investigators (animals/measurements)
MAP, HR	FPI	DeWitt/DeWitt
	CCI Robertson/R	obertson
	EBI DeW	itt/DeWitt
	PBI Rasband/Robertson	
Cerebral perfusion	FPI	DeWitt/DeWitt
	CCI Robertson/R	obertson
	EBI DeW	itt/DeWitt
	PBI Rasband/Robertson	
Vestibulomotor FPI		DeWitt/DeWitt
	CCI Robertson/R	obertson
	EBI DeW	itt/DeWitt
	PBI Rasband/Rasband	
Righting reflexes	FPI	DeWitt/DeWitt
	CCI Robertson/R	obertson
	EBI DeW	itt/DeWitt

	PBI NP	
Memory FPI		DeWitt/DeWitt
	CCI Robertson/R	obertson
	EBI DeW	itt/DeWitt
	PBI Rasband/Rasband	
Anxiety FPI		DeWitt/DeWitt
	CCI Robertson/R	obertson
	EBI DeW	itt/DeWitt
	PBI Rasband/Rasband	
Surviving neurons	FPI	DeWitt/DeWitt
	CCI Robertson/R	obertson
	EBI DeW	itt/DeWitt
	PBI Rasband/Rasband	
Blood-brain barrier	FPI	DeWitt/Grill
	CCI Robertson/G	rill
	EBI DeW	itt/Grill
	PBI Rasband/Grill	
GFAP, APP, BDNF IHC	FPI	DeWitt/Grill
	CCI Robertson/G	rill
	EBI DeW	itt/Grill
	PBI Rasband/Grill	
APP – amyloid precursor protein; BDNF – brain derived neurotrophic factor; CCI – controlled cortical impact; EBI – explosive blast injury; FPI – fluid percussion injury; GFAP – glial fibrillary acidic protein; IHC – immunohistochemical; NP – not practical; PBI – pressure blast injury		

Blast-induced Brain Injury Model Characterization:

David Ritzel, a widely recognized expert on the physics of blast injury, has agreed serve as a consultant for the studies of the effects of blast injury on the brain using the Vanderberg blast device. The Vandenberg device was designed by Dr. DeWitt in consultation with Mr. Edward Vandenberg, an expert gunsmith. The device was constructed by Mr. Vandenberg's company, VandenBerg Customs.

Mr. Ritzel traveled to Galveston 23 – 25 June, 2010 to present a day-long workshop on the physics of blast injury. Several mTBI Consortium members attended the workshop. During his visit, Mr. Ritzel reviewed high-speed digital videos of the Vandenberg device and suggested modifications that will be performed by he and Mr. Steve Parks of ORA, Inc., Fredericksburg, MD. In addition, Mr. Ritzel and Mr. Parks will construct a unique table-top conical blast generator. The device, which can be driven by either compressed helium or an oxyacetylene explosive gas mixture, will be constructed so that only the rat's head is exposed to the effects of blast pressures. When the modifications of the Vandenberg device and the conical blast generators are complete, we will have the only laboratory with the capability to compare and contrast the effects of explosive blast injury produced by the detonation of gunpowder or oxyacetylene with those of blast pressures generated by compressed gas.

Data Type, Collection & Storage:

The M/NWG discussed and made recommendations related to the following questions:
What constitutes "mTBI" for each experimental model?

There was a discussion of the need to identify the primary endpoints that will be used to determine levels of mTBI for each model. A distinction between surrogate (e.g. righting reflexes) and primary (e.g. Fluoro-Jade (FJ) staining) was defined. For the FPI, CCI, and EBI (Vandenberg) models, righting reflex (RR) will be used as a surrogate endpoint for defining mTBI. Dr. Robertson noted that level of consciousness was one of the main determinants of injury level in humans. Since RR is a measure of level of consciousness, it is useful as both a surrogate and primary endpoint for defining mTBI. In addition, investigators may use other primary endpoints that are relevant to their studies to further define mTBI. For example, RR correlates well with numbers of FJ-stained hippocampal neurons, a primary endpoint for defining mild FPI. In contrast, the correlation between RR and FJ staining is not yet known for EBI. For the immunohistochemical measurements made in Dr. Grill's laboratory, mTBI may be defined based on degree of BBB compromise (e.g. albumen staining) or APP expression. The correlation between these endpoints and RR is not yet known. In addition, RR suppression measurements aren't feasible for the PBI model used by Dr. Rasband due to the depth of anesthesia required. Therefore, Dr. Rasband will define mTBI based on BBB compromise or some other histochemical measurement.

2. *Where will the raw data be stored?*

The M/NWG members recommended that the raw data remain with the investigators who collected it. Dr. Miller advised that the Consortium website was not structured for the storage of large quantities of raw laboratory data.

3. *Where are descriptive stats (e.g. mean ± sem, n) stored?*

The M/NWG members recommended that compiled descriptive statistics (e.g. means, standard errors/deviations) for numerical data (e.g. MAP, ICP, CBF) for each model be stored on the Consortium website. Dr. Miller confirmed that the website would be suitable for storage of mean data. The M/NWG recommended that the primary investigators (see table above) will collect, compile and upload descriptive statistics for their models.

4. *What data are shared and where?*

As stated above, descriptive statistics for numerical data will be available on the Consortium website.

5. *Where will images be stored (e.g. IHC, MRI)?*

The large size of the image files precludes storage on the Consortium website, images will remain in the laboratories where they were collected.

6. *Behavioral data:*

Numerical data (e.g. Morris water maze latencies, swim speeds, time in quadrant) from the behavioral assessments would be treated the same as other numerical data.

7. *Non-standard Data Collection and/or Sharing:*

"Non-standard" data are defined as those collected by each investigator as part of his/her individual project. Since each project has specific aims related to the specific hypotheses of that project, data not related to model characterization will be collected by each investigator. These

data will remain in the laboratory in which they were collected. However, should an investigator find that the results of their experiments suggest an additional variable that might prove valuable for model characterization, he/she may recommend that the variable be added to the standard data set. For example, should a particular biomarker prove especially sensitive/specific some aspect of mTBI, the investigator may suggest that the biomarker be added to the standard model characterization data set. The M/NWG would then decide whether to recommend that the biomarker be added to the standard model characterization data set.

Additional Behavioral Assessments

Among the most common and persistent cognitive problems reported by patients with mTBI are deficits in executive functioning and attention. These are difficult functions to evaluate in rodents. In order to develop more effective assessments of executive functioning and attention, Dr. DeWitt been in contact with Robert Hamm, Ph.D. of the Virginia Commonwealth University Medical Center and Tim Schallert, Ph.D. of the University of Texas at Austin. Drs. Hamm and Schallert are internationally respected authorities on behavioral assessments in rodents. Both investigators agreed to consult on measures of higher level cognitive functioning in rodents. In addition, Dr. Hulsebosch has invited Mark Whiting, Ph.D. of Radford University to travel to Galveston to present a workshop and seminar on behavioral assessments in rodents on 5 and 6 August, 2010. Dr. Whiting gave an excellent presentation on novel behavioral measures at the recent National Neurotrauma Symposium in Las Vegas, NV.

KEY RESEARCH ACCOMPLISHMENTS

1. The Model and Neuroprotection Working Groups merged into a single Model/Neuroprotection Working Group (M/NWG).

2. The M/NWG identified a set of physiological, histopathological and behavior variables that will be measured in the four models of mTBI used by the mTBI Consortium investigators.

3. The M/NWG recommended that raw data and images remain in the laboratories in which they were collected. Mean numerical data for the model validation variables (item 2) will be compiled by the investigators who collect the data and made available to all mTBI Consortium investigators via the Consortium website.

4. Dr. DeWitt has been working with Mr. David Ritzel and Dr. Steven Parks to more accurately evaluate and modify the blast wave characteristics of the Vandenberg device.

5. Drs. DeWitt and Hulsebosch have been working with nationally and internationally renowned experts on behavioral assessments in rodents to evaluate and, if necessary, improve the behavioral assessment tasks used by Consortium investigators.

REPORTABLE OUTCOMES

1. A database containing basic physiological, histopathological and behavioral characteristics of the animals models of mTBI used by the investigators of the The Mission Connect Translational MTBI Translational Research Consortium.
2. A novel rodent model of mild, blast-induced brain injury.